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16. ABSTRACT

Biochemical and soil microbiology studies indicate that numerous mechnisms exists for the dehalogenation of both aliphatic and aromatic hydrocarbons. Tests described in this paper demonstrate that enzymatic systems are capable of dehalogenation. The report does not answer the question of the biodegradability of halogenated hydrocarbons, but illustrates the need for more information in order to identify compounds that are likely to undergo enzymatic cleavage of the carbon-halogen bond.

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MICROBIAL DEGRADATION OF HALOGENATED HYDROCARBONS

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INTRODUCTION

Microorganisms are the principal agents responsible for the recycling of carbon in nature. Over geological time, enzyme systems have evolved for the degradation of naturally occurring organic molecules. In fact, the metabolic versatility of microbial populations has led some microbiologists to believe that for every organic compound there exists somewhere a microorganism that will catalyze its degradation. Dagley[1] has discussed this concept as having no basis in fact and nowhere is this more evident than in studies on the microbial degradation of organochlorine compounds. The natural distribution of halogens in terrestrial and marine environments has resulted in the biosynthesis of certain halogenated organic compounds. Thus organochlorides can occur as aromatic, alicyclic, aliphatic and

heterocyclic derivatives. In view of the abundance of bromine in marine environments, it is not surprising to find organobromides are synthesized by marine algae. Many of these compounds are brominated aromatic derivatives.[2] For all of these naturally occurring organohalides it is reasonable to assume the existence of microorganisms that will degrade them to carbon dioxide, water and halide ions. In contrast, the last 30 years have seen the synthesis of enormous quantities of organochlorine compounds. The chemical properties of these molecules have resulted in their use as herbicides, pesticides, solvents, insulators and plasticizers. Many of these compounds are introduced into the environment for a specific purpose, *e.g.*, chlorinated herbicides. Others, such as polychlorinated biphenyls (PCBs), have a wide environmental distribution due to accidental or deliberate deposition coupled with recalcitrance to microbial degradation. Another source of halogenated organic compounds in the environment is the chlorination procedures used in the treatment of potable water, wastewater and cooling water. Jolley[3,4] and Glaze and Henderson[5] have identified the compounds listed in Table I as constituents of chlorinated municipal sewage effluents. Although these compounds are produced in freshwater they could eventually accumulate in the marine environment. In addition, the studies of Sugam and Helz[6] suggest that the chlorinated procedures used for the treatment of sea water or estuarine waters will lead to brominated analogs of the compounds listed in Table I. This is because chlorine readily oxidizes the bromine in sea water and because brominated oxidizing species effect the halogenation of various organic molecules.

Although little is known about the specific halogenated compounds in sea water that result from chlorination procedures, existing information relating to the microbial degradation of organohalides may be used to predict the possible fate of such molecules in marine environments.

Table I. Chloro-Organic Constituents in Chlorinated Municipal Sewage Effluents[a]

Aliphatic Hydrocarbons
- Chloroform[b]
- Dibromochloromethane[b]
- Dichlorobutane, 27 ppb[b]
- 3-Chloro-2-methylbut-1-ene, 285 ppb[b]
- Chlorocyclohexane, 20 ppb[b]

Aromatic Hydrocarbons
- *o*-Dichlorobenzene, 10 ppb[b]
- *p*-Dichlorobenzene, 10 ppb[b]
- Chloroethylbenzene, 21 ppb[b]
- Trichloroethylbenzene, 12 ppb[b]
- Chlorocumene[b]
- Trichlorocumene[b]
- Dichlorotoluene[b]
- Trichloromethylstyrene, 10 ppb[b]
- Tetrachloroethylstyrene[b]

Aromatic Acids and Esters
- 2-Chlorobenzoic acid, 0.3 ppb
- 3-Chlorobenzoic acid, 0.6 ppb
- 4-Chlorobenzoic acid, 1 ppb
- 3-Chloro-4-hydroxybenzoic acid, 1 ppb
- 4-Chloromandelic acid, 1 ppb
- 4-Chloroacetic acid, 0.4 ppb
- 5-Chlorosalicylic acid, 0.2 ppb
- Trichlorophthalate[b]
- Tetrachlorophthalate[b]

Ethers
- Trichlorodimethoxybenzene[b]
- Tetrachloromethoxytoluene, 40 ppb[b]
- Dichloromethoxytoluene, 32 ppb[b]

Ketones
- Tetrachloroacetone, 11 ppb[b]
- Pentachloroacetone, 30 ppb[b]
- Hexachloroacetone, 30 ppb[b]

Phenols
- 4-Chloro-3-methylphenol, 2 ppb
- 2-Chlorophenol, 2 ppb
- 3-Chlorophenol, 0.5 ppb
- 4-Chlorophenol, 1 ppb
- 4-Chlororesorcinol, 1 ppb
- Trichlorophenol[b]
- Tetrachlorophenol, 30 ppb[b]

Nucleosides
- 5-Chlorourdine, 2 ppb

Purines
- 8-Chlorocaffeine, 2 ppb
- 6-Chloro-2-aminopurine, 1 ppb
- 8-Chloroxanthine, 2 ppb

Pyrimidines
- 5-Chlorouracil, 4 ppb

Alcohols
- Chloro-α-methylbenzyl alcohol[b]
- Dichloro-α-methylbenzyl alcohol, 10 ppb[b]
- Trichloro-α-methylbenzyl alcohol, 50 ppb[b]

Amines
- N-Methyltrichloroaniline, 10 ppb[b]

[a]Estimated concentrations in μg/l (PPB) are given for selected constituents.

[b]These compounds are given in Glaze and Henderson.[5] All other compounds are given by Jolley[3] (taken from Jolley[4]).

MECHANISMS USED BY MICROORGANISMS FOR THE DEGRADATION OF HALOGENATED ORGANIC COMPOUNDS

Current theories of microbial evolution suggest that chemically synthesized halogenated molecules will be degraded only if they bear a structural relationship to naturally occurring compounds. Biodegradation of halogenated compounds can only be considered complete when halide ions are released and the carbon skeleton is converted into metabolites common to all living cells.[2]

Unlike many synthetic organic pesticides, halogenated compounds resulting from water chlorination bear some structural similarity to natural products. In effect, these compounds are halogenated natural products. However, the position and number of halogen substitutions can render the compound resistant to microbial attack. Bacterial enzyme systems exist which can effectively dehalogenate naturally occurring organohalide compounds.[7,8] The actions of these enzymes are summarized in Figure 1. The enzyme system, haloacetate halidohydrolase, was isolated from a soil pseudomonad which could utilize fluoroacetate as a sole carbon source. The enzyme system is also capable of dehalogenating chloroacetate and iodoacetate; however, fluoroacetate is the preferred substrate. The enzyme will not catalyze significant release of halide from a variety of other test compounds (Table II). It appears the enzyme is relatively specific for the acetate portion of the molecule. Other enzyme systems (Figure 1) were isolated from bacteria enriched from the aquatic environment capable of growth on dichloroacetate. Halidohydrolase I, when isolated from cells grown on dichloroacetate, will react with monochloroacetate nine times more effectively. On the other hand, under the same conditions, halidohydrolase II was only half as effective in releasing halide from the monochloroacetate. Additional compounds were tested with these enzymes; their range of specificity is shown in Table II. All three systems effect the release of a halide on carbon-2, from compounds of L-configuration possessing a proton substituent on the carbon-2 of the substrate. A number of other systems which have been described seem to conform to this same class

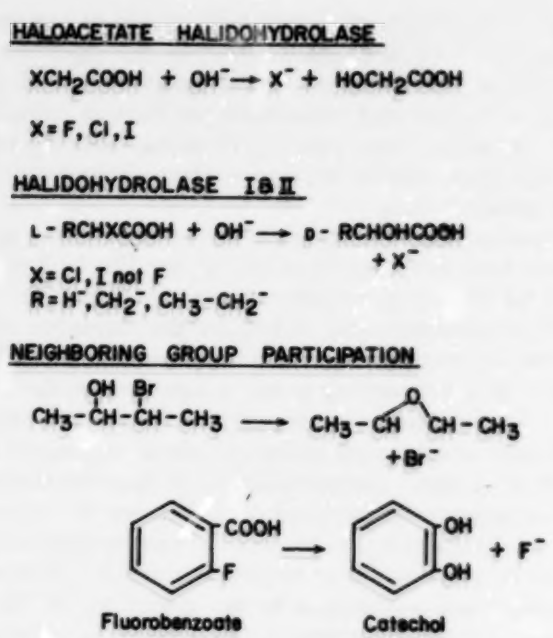


Figure 1. Enzyme systems active in dehalogenation.

Table II. Reactivities of Halidohydrolases[a]

Substrate	Relative Reaction Rates		Haloacetate Halidohydrolase
	Halidohydrolase I	Halidohydrolase II	
Chloroacetate	X	X	X
Dichloroacetate	X	X	- (F)
L-2-Chloropropionate	X	X	- (F)
L-2-Chlorobutyrate	X	X	n.t.
Iodoacetate	X	X	X
Fluoroacetate	-	-	X

[a]From Goldman *et al.*[6]

of reactions. These enzyme systems effectively release halide from the carbon-2 of short-chain acids but not from the carbon-3 of short-chain alcohols. It is possible some of these carbon-2 or -3 halogenated compounds do exist in chlorinated sea water. However, these compounds should pose no problem in persistence since specific enzyme systems shown to exist effectively release halides.

Other dehalogenation reactions involving neighboring group participation have been described for halohydrins.[9] This enzyme, isolated from a *Flavobacterium* sp., catalyzes the sterospecific *trans*-elimination of HBr by the formation of an epoxide with the neighboring hydroxyl group oxygen. Direct participation of a neighboring group is also seen in the defluorination of 2-fluorobenzoate by an enzyme system isolated from a pseudomonad which could utilize the latter compound as a sole source of carbon. Defluorination and decarboxylation reactions are apparently catalyzed by a dioxygenase which results in the production of catechol, an intermediate metabolite common to many aromatic hydrocarbon catabolic pathways. No information was available as to the specificity of this system. The dehalogenation of aromatic hydrocarbons was of major interest when we attempted to predict metabolism of halogenated products of chlorination (Table I).

Another bacterial enzyme system capable of dehalogenation involves phenylalanine hydroxylase. This system releases fluoride or iodide during hydroxylation of the *p*-fluoro- or *p*-iodophenylalanine. However, in the case of chlorine or bromine substitution, dehalogenation is only a minor reaction with the predominant reaction being the hydroxylation-induced migration (NIH shift) of the halogen to the *m*-position and hydroxylation at the *p*-position.[10]

Numerous other enzyme systems for effecting dehalogenation have been characterized in mammalian microsomal fractions as well as insecticide-resistant houseflies. However, these pathways are beyond the scope of this paper and will not be discussed.[7]

A teleological basis exists for the evolution of most of the dehalogenating reactions discussed here. Halogenated natural products can be degraded by the halidohydrolases or haloacetate halidohydrolase. Thus, the organisms bearing these systems

have a selective advantage in the environment. However, it appears many dehalogenating reactions are catalyzed by enzymes normally functioning for other purposes. These adventitious enzyme systems can be considered accidents of nature but, ecologically, they perform a very important function. These enzymes can aid in the detoxification of halogenated compounds in the environment as well as in humans and other animals. Examples of an adventitious enzyme system are phenylalanine hydroxylase and benzoic acid oxygenase. These systems normally function to convert phenylalanine to tyrosine and benzoic acid to catechol. It is probably an accident of nature that the enzyme functions dehalogenate the halogenated substrate analog. A number of adventitious enzyme dehalogenations have been characterized by different investigators (Table III). It is evident from this list that this type of reaction involves a diversity of enzyme reactions and structural characteristics of substrates. Certainly the list of adventitious enzymatic dehalogenation reactions will be much longer when more information is available.

Many other microbiologically mediated dehalogenation reactions have been reported in studies involving the fate of chlorinated pesticides. Most of these studies do not involve characterization of the mechanisms in dehalogenation, and most of the reactions are limited to fortuitous dehalogenations since few of these compounds can be utilized as sole sources of

Table III. Microbial Dehalogenation Reactions

Microorganism	Substrate	Product	Reference
Aspergillus sp.	2-chlorophenoxy-acetate	2-hydroxyphenoxy-acetate	Faulkner[20]
Achromobacter sp.	2,4-dichlorophenol	4-chlorocatechol	Horvath[15]
Arthrobacter sp.	3-chlorobenzoate	3-hydroxybenzoate	Johnston[17]
Clostridium sp.	hexachlorocyclo-hexane	benzene	Allen[21]
Arthrobacter sp.	4-chlorobenzoate	4-hydroxybenzoate	Ruisinger[16]

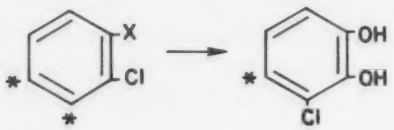
carbon. An extensive review of these reactions is presented by Kearney and Kaufman.[11]

At least two classes of related compounds (chlorobenzoates and chlorophenoxyalkanoates) have been extensively studied both qualitatively and mechanistically. Both these groups of compounds are closely related to compounds found as halogenated by-products of water chlorination (Table I). Studies with benzoate- and phenol-grown soil bacteria have indicated the accumulation of chlorinated catechols when these cells were incubated in the presence of *ortho- meta-* or *para*-chlorine substituted analogs.[12,13] The same types of reactions occurred when soil microorganisms were incubated with chlorobenzoates substituted at two or more carbons.[14] However, in at least two other studies, metabolism of the chlorinated analog was shown to proceed past the chlorocatechol to dehalogenation and mineralization of the substrate.[15,16] These reactions are summarized in Figure 2. The first study[15] suggests a new pathway for halogenated benzoates. There is a direct replacement of the chlorine by a hydroxyl group prior to ring cleavage. In the latter study using 4-chlorobenzoate,[16] results also suggest a direct replacement of chlorine by the hydroxyl group. These studies indicate a dehalogenation reaction similar to that previously mentioned for phenylalanine hydroxylase, suggesting the adventitious dehalogenation by the hydroxylase.

Direct replacement of a chlorine on an aromatic ring by an hydroxyl group has been observed in studies with the fungus *Aspergillus niger* and the bacterium *Achromobacter* sp. (Table III). The uniqueness of certain dehalogenation reactions by microbes within one genus serves to emphasize the specificity of certain enzymes and the structural characteristics that participate in adventitious dehalogenations.

Another group of halogenated compounds (closely related to those in Table I) for which numerous studies have elucidated the metabolic pathways are the chlorinated catechols that result from the breakdown of the chlorophenoxyalkanoates. Figure 3 shows the pathway described for the mineralization of the extensively used herbicide 2,4-D. In the breakdown of 2,4-D, the first step is the cleavage of the ester linkage resulting in the production of the chlorinated catechol. Tiedje *et al.*[18]

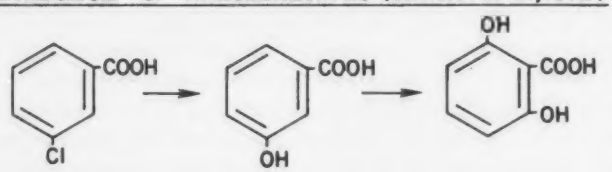
CO-METABOLISM OF HALOBENZOATES (Spokes & Walker, 1974)



Halobenzoate — Halocatechol (Accumulates)

X = -OH, -COOH
* = halogen position

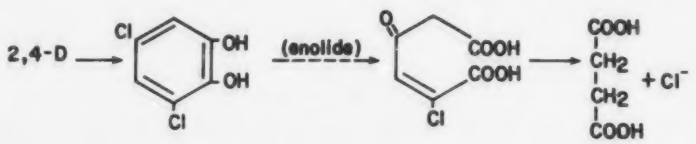
METABOLISM OF HALOBENZOATES (Johnston et al., 1972)



(Ruisinger et al., 1976)

4-chlorobenzoate → p-hydroxybenzoate → protocatechuic acid

Figure 2. Metabolism of chlorobenzoates.



Dichlorocatechol — Chloromaleyl-acetic Acid — Succinic Acid

Figure 3. Halocatechol degradation.

isolated enzymes from an *Arthrobacter* sp. which catalyzed the oxidation of this chlorocatechol by meta fission prior to dehalogenation. However, as metabolism proceeds both chlorines are released, resulting in production of succinic acid, an easily degradable substrate. In this case, the mono- or dichloro-substitution did not inhibit the metabolism of the aromatic ring.

It appears from biochemical and soil microbiology studies summarized here that numerous mechanisms exist for the dehalogenation of both aliphatic and aromatic hydrocarbons. These studies show the existence of enzymatic systems capable of dehalogenation. Whether these studies are applicable to the marine environment remains to be determined. For example, *Arthrobacter*, an organism possessing significant biodegradation potential in soils, is not known to exist in the marine environment. However, if the enzymes evolved in the terrestrial environment it seems feasible to assume the same type reactions could occur in the marine environment where the microorganisms have been exposed to halogenated compounds resulting from marine algae. Other environmental factors, *e.g.*, nutrient levels and concentration, and the degree of substitution probably play a major role in the susceptibility of these halogenated hydrocarbons to microbial attack. Although this summary does not answer the question of biodegradability of halogenated hydrocarbons, it can serve to illustrate the need for more information before predictions can be made regarding which compounds are likely to undergo enzymatic cleavage of the carbon-halogen bond.

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